POSTER PRESENTATIONS

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ADVANCE@MRC Harwell: a new training centre for Laboratory Animal Science and Genetics

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Background

In recent years there has been much publicity surrounding the growing concern around the future shortage of technical skills and expertise across all sectors in the UK. Various factors are thought to contribute to this looming 'Skills Gap', but an ageing technical workforce with many experienced technicians nearing retirement, and a lack of suitable training opportunities are thought to be the main issues that need to be addressed. Additionally, in science and particularly laboratory animal science (LAS), there is very little formal training offered in the UK to support the increase in both the knowledge and technical skills required to deliver reproducible science. Additionally, various discussions have become recurring themes in laboratory animal science and have further highlighted the need to appropriate training.

For example, following the publication of the NC3Rs' Animal Research: Reporting of *in vivo* experiments (ARRIVE) guidelines, there has been an increased demand for improved reporting and, alongside this, there has been an awareness of the need to critically evaluate experimental protocols and biomedical data, with many studies and discussions revealing probable sources of variability. Appropriate training is also a key requirement to help reduce the number of animals used in research while increasing the usefulness of the data generated from *in vivo* models.

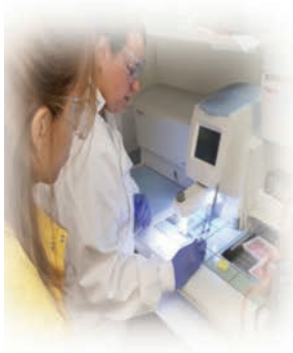
The MRC Harwell Institute is at the international forefront of the advancement of medicine and knowledge through the discovery and investigation of mouse models of human disease. The Institute recently secured funding to construct a new training centre, Advance@MRC Harwell, with versatile laboratory, conferencing and IT training spaces, set to open in 2020. The new training centre will make an important contribution to scientific training in the UK. With animal welfare being a continuous theme through the development and delivery of training courses at MRC Harwell. The existing training programme is focussed

Figure 1. Architects representation of finished building.

on mouse genetics, *in vivo* skills, ethics and welfare, laboratory and technical skills as well as experimental design and statistics. In order to expand the range of courses to be offered through the training centre, Advance@MRC Harwell is forging links with a growing network of training providers, including academic organisations, societies and commercial firms who are keen to collaborate and utilise space and facilities in the training centre.

Key training divisions

Wet Laboratory * Surgical Training * IT * Bioinformatics * Conferencing * Breakout Spaces * Webinar



Current courses available

- Entry level mouse genetics for animal technicians and junior researchers.
- Advanced mouse genetics for colony managers and senior researchers.
- Genome editing using CRISPR/Cas9 in the mouse.
- Practical training course in transgenic technology.
- Mouse embryo and sperm cryopreservation.
- Mouse necropsy tissue/cut-in and processing.
- Collection and processing of blood and urine samples from mice.
- Pipetting, weighing and dosing skills for mouse husbandry staff.
- An introduction to histology.
- An introduction to light microscopy.
- An introduction to proteomics.
- Procedural individual licence Modules A, B and C.

For more information about training courses and the use of training spaces email:
mlc-training@har.mrc.ac.uk

Competency assessment for skill acquisition: The TASK Model

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Introduction

Charles River is a large global corporation with many employees located in various locations across the globe. This presented a challenge in ensuring consistency within our company, as there are cultural differences between countries, different animal production facilities as well as scientific sites that carry out very different types of studies, both *in vivo* and *in vitro.* All of our sites have training programmes in place to meet the needs for that specific site, which has increased workflow and respects the 3Rs. However we realised that we still needed to harmonise many of the common procedures to maintain scientific integrity and reduce any variables regardless of where the study or work was being performed. To meet this global challenge, our competency model Technical Assessment of Skills and Knowledge, known as 'TASK', was developed in order to assess and document the skills of each employee. This document can then be archived in our Learning Management System (LMS) known as www.charlesrivercampus.com and updated as needed based upon new scientific discoveries, development of new assays and updated regulatory compliance requirements.

Technical assessment of skills and knowledge (TASK)

The form shown is a sample of a TASK document that provides an organised approach to the evaluations of a skill set for the subcutaneous cell line engraftment technique. Criterion is listed in which our employees were initially scored when they performed their practice subcutaneous cell line engraftments. Once the employee successfully completes the competency criterion which is a qualitative assessment with specific skill sets that are defined, the learner must demonstrate the skill to a trainer who then records observations. To demonstrate proficiency, which is a high degree of skill, the employee must perform an internal N=100 animal study with tumour growth achieving 50% or above the specified start size range while under the direction of a trainer or SME. These standards are a set of quantitative measurements. These specific skill sets and quantitative assessments are how the parameters are set that are then used to evaluate an employee against a TASK performance scale.

TASK Name: Subcutaneous Cell Engraftment

	Competency criterion (check each criterion if evident or not evident evident during assessment)	Evident	Not evident
1	Demonstrates drawing up the cell suspension		
2	Identifies correct injection target sites: central right flank		
3	Demonstrates proper restraint: mice are held gently but firmly by the scruff, mouse does not exhibit signs of struggling or difficulty in breathing.		
4	Describes and demonstrates subcutaneous injection of cell suspension, needle is bevel up before placed into the subcutaneous space, skin is tented with the needle to confirm needle depth is appropriate (tip moves freely), appropriate amount of cell suspension is delivered to correct target site.		
5	Demonstrates proper needle removal: needle is rotated clockwise during removal to mitigate cell suspension leakage from injection site.		

Proficiency criterion (check each criterion if achieved or not achieved during assessment)		Evident	Not evident
1	Reviews applicable SOPs and regulations.		
2	Attends lecture/didactic training on subcutaneous injections in the mouse.		
3	Properly answers questions regarding target engraftment as described in the protocol.		
4	Demonstrates adequate knowledge of dosage limitation and troubleshooting, performs adequate safety awareness/measures, documentation with LIMS system, and adherence to all SOPs for all procedures with the mouse.		
5	Demonstrates tumour engraftment proficiency in an internal practice.		

Figure 1. Example of TASK document.

TASK Performance Scale

The TASK method is a process that addresses defined area(s) of work, defined skill sets, competency and proficiency standards.

Figure 2. TASK performance scale.

Project analytics

Project Analytics

Model (n)	Line	Target Range	Engraftment Duration in Days (Std. Deviation)	Range Accuracy
4T1 (21)	Breast	50 - 100 mm^3	11.2 (2.3)	100.0%
MC38 (21)	Colon	50 - 100 mm^3	11.1 (2.1)	97.2%
CT26 (18)	Colon	50 - 100 mm^3	10.9 (1.1)	100.0%
LL (6)	Lung	50 - 100 mm^3	12.0 (2.3)	100.0%
B16F10 (31)	Melanoma	50 - 100 mm^3	8.5 (1.6)	95.7%
A549 (10)	Lung	100 - 150 mm^3	23.4 (2.4)	97.6%

Figure 3. The data above highlights standard oncology cell lines and its projected growth overtime with a tight standard deviation and highly reliable adherence to a targeted range.

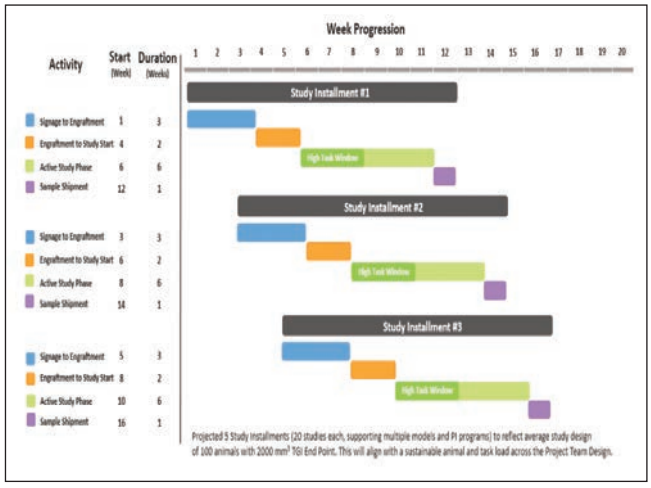

Figure 4. The data then shows our high throughput capabilities via a Gantt chart which details project milestones and trigger points for overlapping the work without creating resourcing bottleneck.

Conclusions

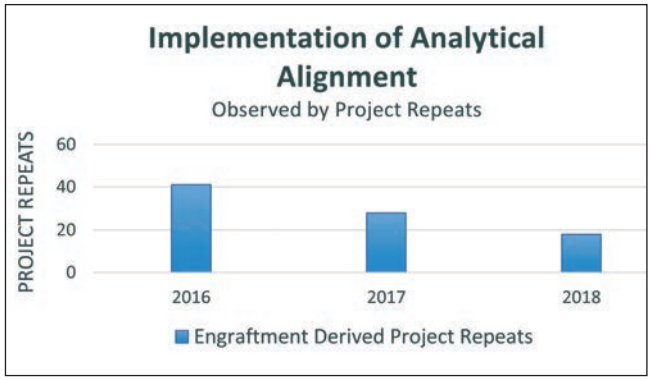

Figure 5. Implementation of analytical alignment.

The application of the TASK Form provided standardisation of the cell line implantation technique and therefore a prediction tool for the success of the study. This performance scale provided a tracking method to show improved technical proficiency which then resulted in less study repeats and reduced animal redundancy by not having to re-engraft a study, a welfare improvement. The demonstrated improved model performance and improved timeline accuracy for *in vivo* high throughput modelling.

- **Developmental:** The TASK form for the engraftment technique was introduced by the Research Manager as an evaluation for proficiency and is also integrated into the technician's annual performance review in the form of a numeric metric evaluation.
- **Resourcing Logistics:** The TASK model allows for improved visibility in aligning technical proficiency of a research technician to model specific projects. By having a set of metrics to evaluate the technician, a foundation of standardisation was established as mechanisms of quality control and data integrity.

- **Leadership:** A clear assessment of technical skills quantifies technical proficiency upstream to identify trainers for model specific applications. Statistically tracking and identifying successful implanters will provide an opportunity to place them in training roles to teach the nuances of implanting certain cell lines.
- **Reduction of Internal Resources:** By use of the TASK model of competency and proficiency, we were able to reduce the number of repeat studies (Figure 3), which also translated into reduction of consumables and efficient use of internal resources over time.
- **3Rs:** A key outcome emerged in that we were able to recognize two of the key components of the 3Rs.
  1. Reduction in the number of animals placed on studies.
  2. Refinement due to proper training in techniques in order to alleviate or minimize pain and distress while animals are on a study.

Acknowledgements

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E-Learning: a flexible learning solution for an ever-changing world of work

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Introduction

In an ever-changing and increasingly competitive world of work where globalisation has led to greater diversity in the workplace, employers must come to terms with a new environment in which flexibility plays a leading role in attracting, retaining and developing staff. With an estimated 92% of the millennial generation identifying flexibility as a top priority when job hunting, it is time for organisations to evolve to meet the needs of an agile, digital, focussed and flexible workforce.[1]

A changing world of work

Meeting the development needs of individuals in an ever-growing workforce, in increasingly demanding work environments can prove challenging and costly.

Organisations are faced with the complexity of combining the need for a stable, competent and present workforce with increasingly flexible working solutions, that do not impact on opportunities for growth, development and career progression.

Adopting a flexible working environment is known to have associated benefits of increased efficiency, motivation, staff retention and improved employee relationships (Department of Business and Skills Staff 2014) but this presents challenges associated with providing equal opportunities for career progression and continuing professional development.[2]

Training and CPD

Within the research industry, the changes to The Animals (Scientific Procedures) Act 1986 (as amended in 2012), with the additional responsibilities for ensuring staff are educated, competent and continuously trained, highlights the need for a new approach to training and (Continuing Professional Development) CPD in order to attract, develop and retain skilled Animal Technologists and to support their career pathways.[3]

With the 3Rs at the heart of the Institute of Animal Technology (IAT) Career Pathway, investing in staff development remains essential in promoting excellence in animal care and welfare and in supporting good science.

Whilst structured learning is far from obsolete and remains fundamental to learning and development strategies; with learners desiring an increasingly flexible and accessible learning culture, traditional face-to-face programmes need to evolve.

Meeting these challenges involves a balancing act of breaking with the past and being ready.

The future of learning

New working practices, emerging technologies, dispersed locations and a multi-generational workforce are directly influencing the design and delivery of learning, with a 50% increase in the use of technology in learning and development over the last five years.

Research shows top performing organisations are using technology to increase the effectiveness of formal learning; this flexibility, desired by 94% of learners today, also realises real business benefits, as they are:

- 3 x more likely to report an increase in job productivity
- 2 x more likely to improve staff retention
- 3 x more likely to improve organisational performance

Blended learning defines the integration of both traditional face-to-face learning and online methods of learning delivery (Towards Maturity Staff 2018) transcending the limitations of a single method of learning delivery and providing learners with options to expand their knowledge at their own pace, at a time suitable to them.

The prevalence of e-learning has grown rapidly, with corporate e-learning increasing by 900% in the past 16 years (Elearning Industry Staff 2017), which is unsurprising when considering the recognised benefits of:[4]

– Flexibility of access from anywhere at anytime.
– Ability to reach simultaneously an unlimited number of employees.
– Consistency of delivery of training and learning.
– Potential to achieve cost reductions/cost effectiveness.
– Ability to log or track learning activities.

E-learning provides opportunities for all, with multi-media methodologies to suit most learning preferences. With online communities and discussion groups to further support a new generation of technically competent and self-directed learners, it is time for organisations to embrace and incorporate e-learning into their learning and development strategies to provide a modern, flexible and fully inclusive approach to learning, and to meet the needs of an ever changing and evolving workforce.

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A sweet change to the needle

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Background

Some of our rats undergo mini pump surgery. This is a procedure in which a device is implanted under the skin of an animal and catheterised into a femoral artery for intravenous infusion. This device or "mini pump" then provides a slow, constant release of compound over a specific time and can be used to replicate human PK profiles. The mini pump replaces the need for repeated Subcutaneous (s.c.), Intraperitoneal (i.p.) or Intravenous (i.v.) V injections. In this instance, it was replacing the need for multiple i.v. injections.

The preoperative analgesia that the animals receive is an injection of Carprofen and an injection of local anaesthetic at the incision sites. This analgesia is given when the animal is being prepared for surgery whilst under anaesthesia.

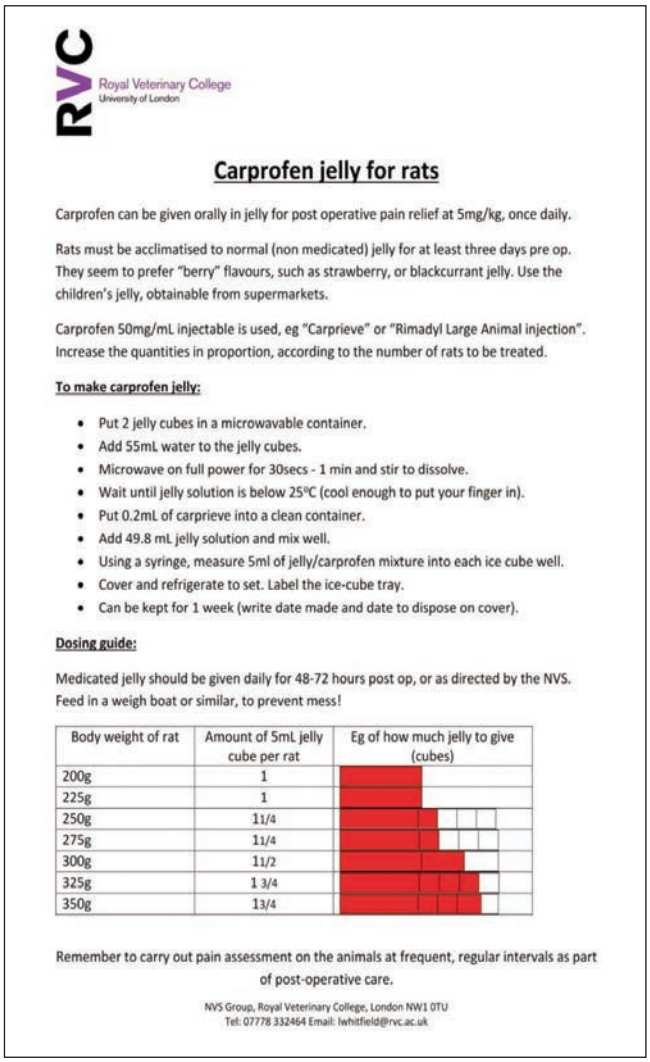
The post-operative analgesia that they received was a s.c. injection of Carprofen once a day for three days after surgery. However, our Named Veterinary Surgeon (NVS) suggested providing the pain relief in flavoured jelly to avoid the use of needles.

Method

To acclimatise our rats to the taste and texture of the jelly and to ensure they would happily consume the jelly when needed, we made up batches of non-medicated jelly to try them with first. We provided this non-medicated jelly for three days before the medicated jelly was introduced.

Figure 1. Rats eating unmedicated jelly.

The Royal Veterinary College provides a fantastic recipe that can be used to make as little or as much medicated jelly as needed.



Royal Veterinary College
University of London

Carprofen jelly for rats

Carprofen can be given orally in jelly for post operative pain relief at 5mg/kg, once daily.

Rats must be acclimatised to normal (non medicated) jelly for at least three days pre op. They seem to prefer "berry" flavours, such as strawberry, or blackcurrant jelly. Use the children's jelly, obtainable from supermarkets.

Carprofen 50mg/mL injectable is used, eg "Carprieve" or "Rimadyl Large Animal injection". Increase the quantities in proportion, according to the number of rats to be treated.

To make carprofen jelly:

- Put 2 jelly cubes in a microwavable container.
- Add 55mL water to the jelly cubes.
- Microwave on full power for 30secs - 1 min and stir to dissolve.
- Wait until jelly solution is below 25ºC (cool enough to put your finger in).
- Put 0.2mL of carprieve into a clean container.
- Add 49.8 mL jelly solution and mix well.
- Using a syringe, measure 5ml of jelly/carprofen mixture into each ice cube well.
- Cover and refrigerate to set. Label the ice-cube tray.
- Can be kept for 1 week (write date made and date to dispose on cover).

Dosing guide:

Medicated jelly should be given daily for 48-72 hours post op, or as directed by the NVS. Feed in a weigh boat or similar, to prevent mess!

Body weight of rat	Amount of 5mL jelly cube per rat	Eg of how much jelly to give (cubes)
200g	1	
225g	1	
250g	1 1/4	
275g	1 1/4	
300g	1 1/2	
325g	1 3/4	
350g	1 3/4	

Remember to carry out pain assessment on the animals at frequent, regular intervals as part of post-operative care.

NVS Group, Royal Veterinary College, London NW1 0TU
Tel: 07778 332464 Email: lwhitfield@rvc.ac.uk

Figure 2. Recipe sheet for Carprofen jelly from the Royal Veterinary College.

Figure 3. Equipment required for feeding analgesic in jelly.

Figure 4. Jelly 'served' in weighing boats.

Figure 5. Rat eating Carprofen medicated jelly.

As our animals were housed individually after surgery, there were no issues with making sure each rat received the correct amount of jelly. However if animals were multiply housed, the animals would need to be separated whilst eating their jelly portions.

We carried out regular welfare checks to ensure the pain relief was just as effective as when given via an injection.

More jelly could also be provided if the animals appeared in any discomfort. It was advised to provide an extra ½ a tray if needed. Fortunately, none of our animals showed any signs of pain or distress and did not need a top-up of analgesia.

The raspberry jelly was their favourite flavour.

Benefits to using jelly instead of an injection:

- no painful needles
- no need to restrain animals
- reducing handling, pain and stress levels can improve recovery time

Figure 6. On the first day, the rats took a few moments before trying the unusual item in their cage.

Figure 7. However, once they discovered it was in fact a wonderful, tasty treat, there was no longer any hesitation when more appeared the next day.

- Less stressful as restraining an animal and giving a painful injection can increase levels of stress.
- Incisions are less likely to become damaged as restraining an animal could cause pulling and/or tightening on the incision site which can be painful for the animal.
- Jelly is a delicious treat for rats and is a nice, positive experience for the animals.
- Technologists who administer the analgesia much prefer giving a tray of jelly instead of having to restrain and inject an animal.

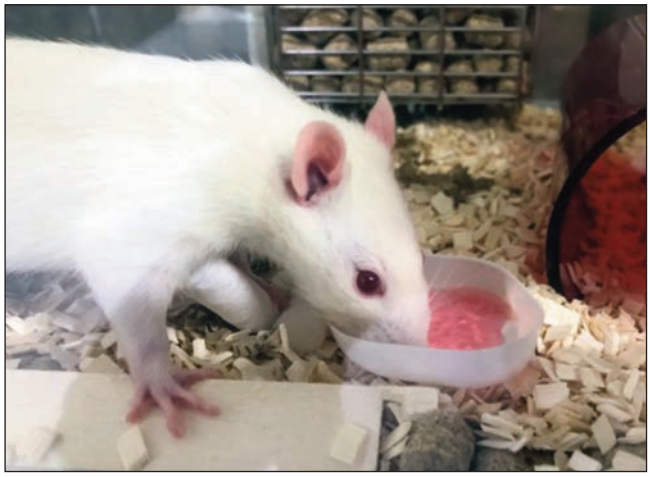


Figure 8. The raspberry jelly was their favourite flavour.

Alternative handling techniques to reduce anxiety in laboratory mice

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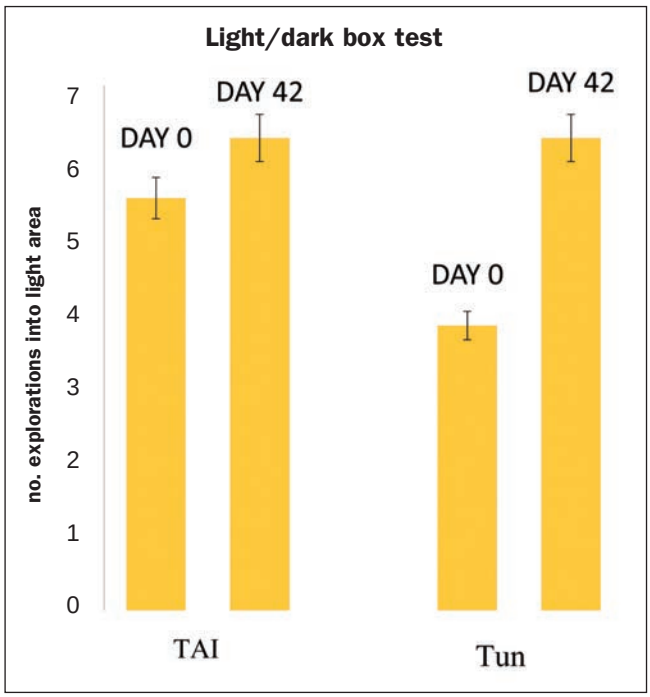
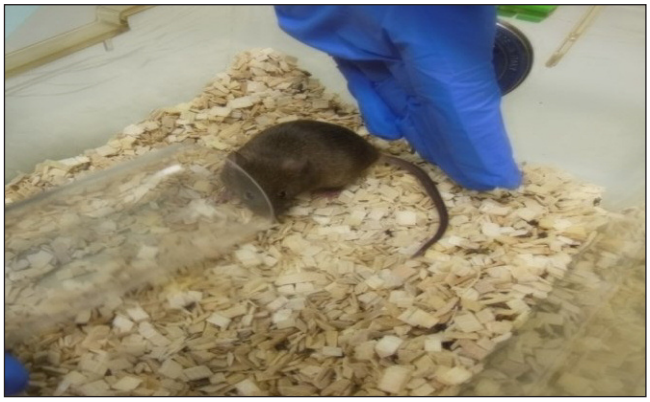
Introduction and aims

Animal Technologists routinely handle and restrain mice as part of their daily duties but the effects of this interaction are often not taken into consideration when designing experimental programmes. The most common and widely used method to capture and transfer mice from cage to cage is to pick up and restrain the mouse by its tail.

Recent studies at Liverpool University, however, have indicated that handling mice by their tails during routine cleaning and procedures induced aversion and high anxiety in many commonly used strains.[1] The evidence from the Liverpool study suggests that habituating the mice to use a clear plastic tube enables the technologist to move the mice from cage to cage via the tube reducing the need to handle them which in turn lowers anxious behaviours. A clear tube is preferred to allow essential health monitoring of each animal and colour has been found to not be a factor.

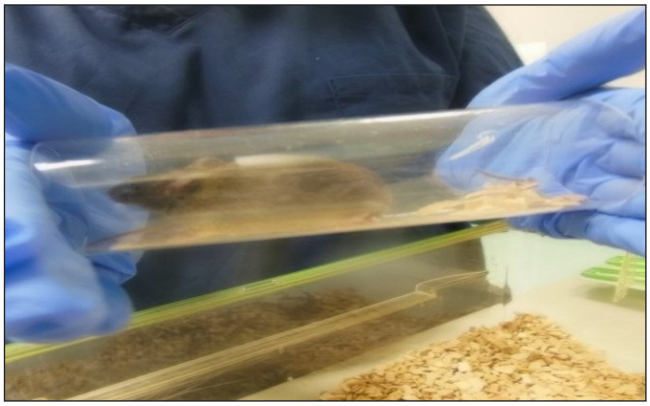


Graph 1. Light/dark box test results.

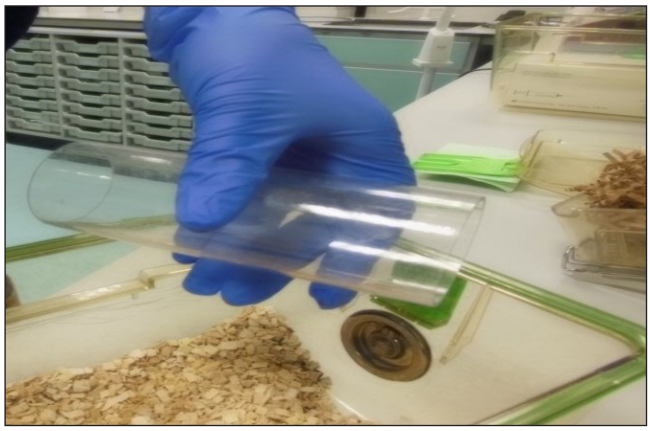


Figures 1 and 2. Mouse tubing technique – mouse entering tube.

We decided to trial this new method to see how long it would take the mice to habituate to the tube and



whether there was any visible reduction in anxiety compared to our normal tail handling method. We were also wanted to assess the time impact that using this alternative method of handling would have on the duties of the animal technician.



Figures 3 and 4. Mouse being removed in tube to clean cage.

Methods

Behavioural testing. All mice underwent light/dark box exploratory test, to evaluate anxiety levels at the beginning of the experiment and 6 weeks later at the end and values compared.[2,3]

Animals. All mice were aged 12 to 16 weeks old (adult and sexually mature) and were group-housed on a 12/12 light dark cycle with access to food and water *ad libitum*, relative humidity 40-55%.

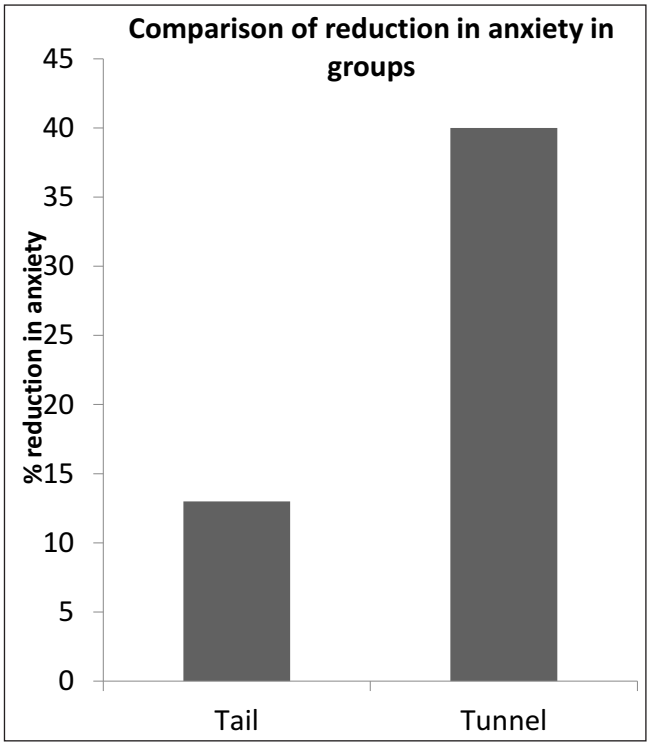
Day one: 12 transgenic male mice on a mixed background were individually placed in a dark box at day 1 and left to acclimatise for two minutes. Once acclimatised the mice were left for another 5 minutes and allowed to explore freely outside the box which was illuminated with a bright light.

After the light/dark test had been carried out 6 mice were given the clear plastic tubes plus standard enrichment in their cage and the other 6 had just standard enrichment.

Once a week at the weekly cleaning, the tube method was used on the six mice that had tubes in their cage and the other 6 mice were cleaned by the 'normal' tailing method.

After the six weeks of using both types of handling the light/dark test was repeated but this time 'blind' so the results were without bias.

Statistical analysis. Differences between means were analysed using the Students paired t-test and were considered different when probability values were less than 0.05.

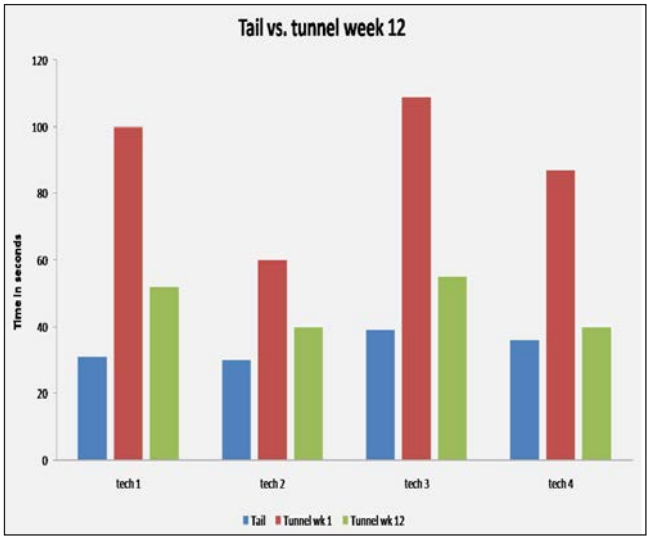


Graph 2. Comparison of reduction in anxiety in groups.

Results

Although the results from the light/dark test comparing each group for anxiety at day one compared to 6 weeks later after the different handling techniques were not statistically significant after analysis (see Graph 1), there is a significant reduction in anxiety (40%) in the group using the tube method. (Graph 2).

During this short trial it was noted the mice using the tube method after a few weeks were willing to enter the tube on their own accord and would quite comfortably sit in the tube whilst being transferred. We also observed, consistent with the original publication, that



Graph 3. Time taken by mouse to enter tube.

they preferred their own home cage tube as opposed to a clean one.

We also observed that the mice being picked up by the tail remained evasive and resisted capture.

During the trial we timed how long it took to clean cages using both methods. Although using the tunnel technique whilst cleaning took longer than handling mice by the tail, by the 12th week the time taken was reduced by 50%. Graph 3 shows the difference in cleaning times for each handling method on week one and week 12.

WEEK 12: THE TAIL METHOD – average 10.10 seconds
WEEK 12: THE TUBE METHOD – average 12.5 seconds

Conclusions

The tube group showed a much greater reduction in levels of anxiety after the 6-week period in comparison to the tail handled group. However, this was not statistically significant upon analysis but may well be if the study was longer or involved more animals. It should also be noted that only males were tested and it would be important to test both sexes since it is well documented that they exhibit different behaviours and response to certain stress factors.

There was a slight decrease in anxiety observed in the tail handled group from day one compared with 6 weeks later, this could possibly be down to memory retention of pre-exposure to the light/dark box.

In summary, the overall reduction in observed anxiety in the tube group combined with the bio containment benefits it offers makes this method worth considering or exploring further given that we found the difference in time taken for each method was negligible when cleaning out mice.

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A technician's guide to ferret enrichment

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Introduction

At the Royal Veterinary College (RVC), we have a colony of ferrets that are used in research and also for training students and delegates. Ferrets are highly intelligent social animals which we house in groups. As they age Ferret behaviour changes as well as with new experiences. In the wild they will spend the majority of their time sleeping and are crespuscular, that is more active at dusk and dawn. Even though they are considered small animals in research, their behaviours are comparable with cats and dogs.

It is therefore extremely important that ferrets in captivity are provided with environments and experiences that allow them to exhibit their natural behaviours. As Animal Technologists we have the responsibility of not only providing basic husbandry requirements such as food, water, shelter and veterinary care but also solutions for their mental well-being. An important aspect of good animal welfare is to provide our animals with appropriate enrichment to allow them to fulfil their natural behaviours.

Behavioural enrichment is a fundamental animal husbandry principle that seeks to enhance the quality of animals in our care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological wellbeing. Enrichment comes in many forms from interactions with fellow animals and technologists to novel interactive toys.

As Animal Technologists we are constantly looking for new ways to improve the welfare of ferrets in our care.

Signs of behavioural problems in ferrets

If ferrets are not provided with the appropriate environment, they may develop behavioural problems. Stereotypic behaviour is one indicator of behavioural issues in your animals.

A stereotypy is normally a repetitive or ritualistic movement or posture.

In ferret's abnormal behaviours include:

- cage circling
- overgrooming
- repetitive behaviours such as bar licking
- lack of interaction with environment
- sitting in the corner of a cage
- no interest in interaction with the cage mates or technician
- aggression towards pen mates

These abnormal behaviours may be due to boredom but we must also consider behaviours of ferrets in pain or distress and any procedures they may have had. For example, if an animal has had a surgical procedure then we may expect to see some of the behaviours above.

Figure 1. Ferret in pen showing examples of enrichment.

Enrichment recommendations

At the Royal Veterinary College, we have carried out an investigation to look at various types of enrichment for our ferrets. Below are some examples of successful enrichment ideas:

– Enrichment does not have to be expensive and we have found even providing a brown paper bag or cardboard box allows the ferrets to interact by chewing and tearing the item.
– Tunnels are a great way to encourage them to

Figure 2. Ferrets interacting with pen mates.

exercise but also provides a place to hide and explore. At the RVC we have tunnels which are mounted to the walls to introduce different heights and levels for extra exploration.
- Sandpits encourage them to dig and forage.
- Paddling pools have been introduced to allow the ferrets to swim and splash.
- Hammocks provide a cosy place for them to sleep and or relax and provides a safe place to hide.
- Ladders are popular allowing the ferrets to climb around the caging and creates different levels and viewpoints for the animals.
- Socialisation is very important and at the RVC we have a programme that involves animal-animal play time and also human animal interaction and handling. This not only provides enrichment but is a valuable training tool for research and teaching purposes.
- Play pen socialisation – animals are removed from their home cages and placed in a floor pen with toys and interaction with technicians.
- Novel edible treats to experience new textures and tastes.

Figure 3. Cages linked using tunnels so that ferrets can move between cages.

Figure 4. View of ferret in tunnel from below.

- Group housing – when they are in groups, they create a sense-based hierarchy and learn how to socialise with other ferrets. This is important as not all ferrets get along. Usually when a ferret is very excited or in a playful mood, they communicate with one another using their 'dook' noise. It sounds like they are laughing to one another. For example, with some noises being quite low in pitch while if they are running around chasing each other it seems to be quite high pitched.
- Cage interlink tubes – these have proved extremely popular with our ferrets and gives them the ability to choose their environment or cage. We can hide treats in the tubes to encourage natural foraging behaviour.

Now and the future

Studies at the RVC have shown that regular interactions with technicians are vitally important to decrease stress levels in the laboratory setting. This not only improves animal welfare but also health and safety when handling for both the animal and the handler.

Animals that are handled regularly and habituated to the laboratory environment provide much better scientific data.

When providing enrichment for our ferrets it is also important to ensure that we do not over stimulate the animals or even cause distress by introducing too many novel environments or experiences in a short period of time or quantity.

Our next project aims to answer the question – How much enrichment is too much?

Figure 5. Human animal interaction and handling.

Figure 6. Group housed ferrets.

Fun ferret facts

- An entire female is known as 'Jill' while a spayed female is called 'Sprite'.
- A new-born ferret is so small it can fit on a teaspoon!
- A group of ferrets is called a 'Business'.
- Ferrets are the third popular pet in the USA!
- The word 'ferret' is from the Latin meaning 'little thief'.
- When ferrets are excited, they perform what is called a 'weasel war dance'.
- Baby ferrets are born both deaf and blind and begin to hear and see at around 34 days old.

Ferret influenza work at The Francis Crick Institute

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Abstract

In conjunction with the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza, ferrets are used at The Francis Crick Institute for the purpose of studying the Influenza virus, focussing on understanding the characteristics of circulating influenza in humans and animals for future vaccine development.

This poster looks at the processes in place starting with the arrival of the ferrets from the Schedule 2 supplier, through the acclimatisation period and the infection procedure until reaching the end point of the study. It also looks at the precautions in place to mitigate against risk of transmission to humans once the ferrets are infected.

Figure 1. Ferret and handler.

Introduction

Influenza viruses continue to affect human and animal health; each year seasonal influenza has a considerable impact on human health and influenza viruses also circulate widely in animal populations posing a zoonotic threat to humans, as seen in the H5N1 avian influenza virus.

The Francis Crick Institute carries out the majority of influenza work under containment level 2 facilities but we do have a containment level 4 suite to use when it is required. The Biological Research Facility (BRF) carries out all husbandry and procedural work for the Collaborating Centre for Reference and Research on Influenza based at The Crick, who then pass their results onto the World Health Organisation (WHO).

Ferrets are intelligent, lively and playful animals even though they can spend up to 75% of their time asleep. They like to explore, play and burrow, so their housing and environmental enrichment should reflect this to allow them to express natural behaviours.

Figure 2. In and out the rabbit ball – Pop goes the Ferret!

Environmental refinements

The ferret holding room is designated for ferret use only, as they are classed as a predator species and strong scents will cause stress for the other animals housed within the unit. Only designated, trained staff members are allowed in the holding room and there is a strict workflow order. Once work has been completed, the Animal Technologist is required to have a full change of clothes before entering the holding rooms housing other species within the unit.

The ferrets are housed in individual pens to help minimise cross infection between virus strains which may otherwise be caused by sneezing.

The pens we use were designed by the BRF team, so are unique to The Francis Crick Institute. The 3Rs were considered in the design; they were refined from the original pens to include a height difference, greater floor space and additional enrichment products.

Figure 3. Ferret pens at The Francis Crick Institute.

They have high clear transparent sides to allow the ferrets to see each other, stand fully upright and jump. The pen size exceeds current minimum standards set out in the Animals (Scientific Procedures) Act 1986, Code of Practice Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific for Scientific Purposes (CoP).

The ramp gives the ferrets the opportunity to make dens by allowing them to hide when they want to but also gives them access to different height levels within the pens.

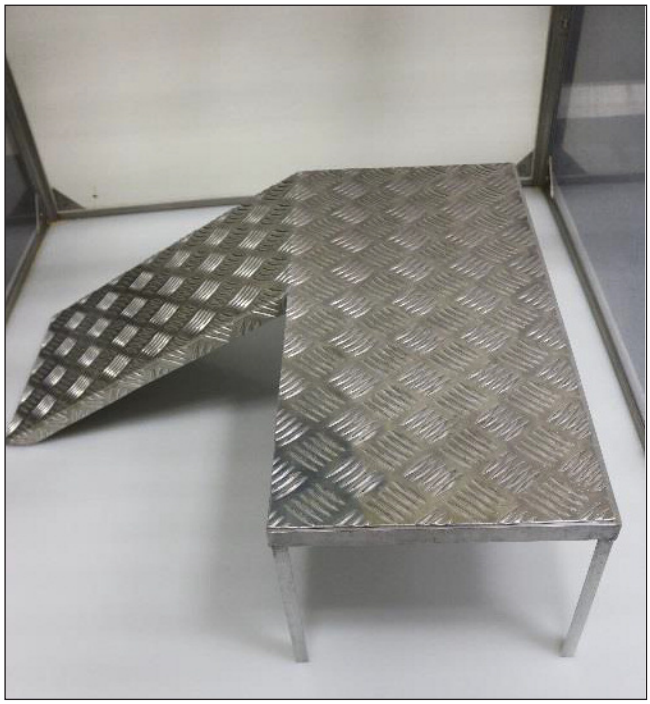


Figure 4. Ramp used by ferret for various activities.

Enrichment

Each pen has its own environmental enrichment.

Figure 5. Pen showing ferret utilising ramp and environmental enrichment.

Figures 6-8. Examples of the enrichment provided.

The pens are filled with dust-free wood shavings to a depth that allows them to burrow and play. They are given a large rabbit ball and tube that enables them to exhibit natural behaviours such burrowing and hiding. They are also given a smaller ball that has a bell inside which they can move round the cage. The enrichment balls are rotated on a weekly basis to help keep the ferrets engaged.

Figure 9. Ferret pen showing environmental enrichment.

Before infection, the ferrets have playtime outside of their pens. On a rotating system, the ferrets are let out individually to explore the holding room and interact with the staff. This is good exercise for them and it can help with the up-take of the virus and can assist in a quicker terminal bleed (bleed-out) time. A radio is also on timer during the day to provide background noise for the ferrets.

Figure 10. Ferret exploring during play time.

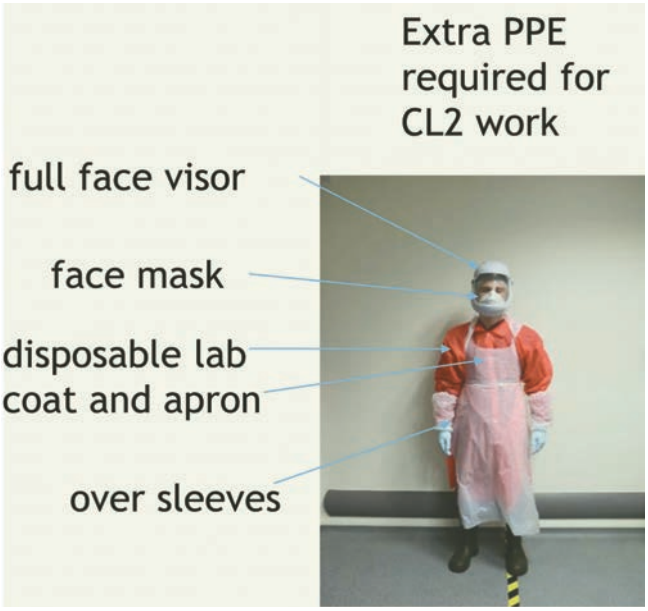
Ferrets are intelligent, lively and playful animals even though they can spend up to 75% of their time asleep. They like to explore, play and burrow so their housing and environmental enrichment should reflect this to allow them to express natural behaviours.

Diet

Ferrets are carnivores so they are fed LabDiet 5L7D high density Irradiated pellets *ad libitum*. This is high protein/high fat food, as required for the ferrets to function effectively. After infection, diet supplements may be given if ferrets are observed as being lethargic or show loss of appetite.

Figure 11. Ferret diet supplements.

Procedures



Day one ferret arrival

Each ferret's weight is recorded and they are then placed into their pens where they are allowed to acclimatise for 1 week.

Day 7 infection day

1. Food is removed at least 3 hours prior to the procedure to avoid the ferrets vomiting when anaesthetised.
2. The ferrets are then re-weighed pre-infection in order to monitor weight.
3. Technologist 1 will remove the ferret from the pen and place into the anaesthetic chamber where they are given an isoflurane/oxygen mix for 6 minutes and monitored to make sure they are slowly going to sleep.
4. They are anaesthetised as otherwise we would have to restrain the ferret, this in turn would cause more stress and the ferret is more likely to sneeze. This way a smaller volume of virus can be administered.
5. Technologist 2 prepares the viruses; each ferret is given the total of 1ml administered by the intra-nasal route.
6. The ferret is removed from the chamber and placed onto the bench top on its back. Technologist 1 will administer the virus, whilst person 2 holds the ferret and monitors breathing and uptake of the virus.
7. Once the procedure is complete, the ferrets are monitored for their righting reflex before being taken back to the pen. The ferrets recover very quickly from the procedure and will be back to their lively selves within a few minutes.
8. After each infection the bench is wiped down with a sodium hyperchlorite dilution and the disposable apron, over sleeves and gloves are disposed of appropriately.

The process can then be repeated for each ferret, each time changing disposable PPE to avoid cross infection with the virus strains.

Day 7-21 monitor

– The ferrets are monitored twice daily post infection for the onset of influenza; clinical signs can include bouts of sneezing, mucous nasal discharge, lethargy, conjunctivitis and photophobia.
– Clinical signs normally appear 48 hours post infection. If any unexpected signs are noted the (Named Veterinary Surgeon) NVS will be called for advice.

Day 21 terminal bleed

– The ferrets are weighed in the holding room prior to bleed-out so that the anaesthetic dose volume can be calculated. The bleed-out will be performed in a designated procedure room.
– The anaesthetic is then mixed ready for injection.

Equipment is prepared in the procedure room, for each ferret the following equipment is required:

- syringe pre-filled with pentobarbital
- clinical waste bag

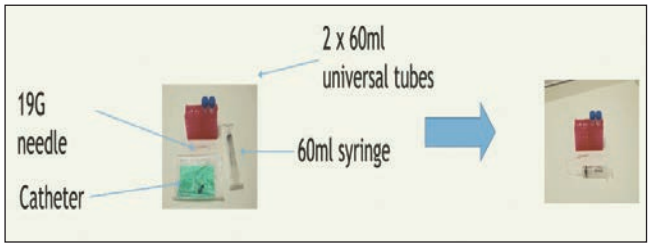


Figure 12. Equipment required for terminal bleed.

Technologist 1 will retrieve the ferret from the holding room and bring to the procedure room.

1. The ferret will then be placed on the work bench and restrained, whilst Technologist 2 will perform an IM injection of anaesthetic.
2. Once the ferret is asleep, it will be placed onto its back.
3. Technologist 1 will then palpate around the heart, feeling for the strongest heartbeat; once found the needle can be inserted into the heart.
4. Technologist 2 will be holding the syringe; when it starts to fill with blood, they will draw back slowly until the required amount is obtained. Sometimes the needle may need repositioning as the heart moves during the procedure and the blood flow may also slow down or stop momentarily whilst the heart re-fills with blood.
5. When the required amount is achieved (no less than 50ml), the syringe will be removed from the needle and replaced with the pentobarbital filled syringe to administer an overdose of anaesthetic.
6. Once heartbeat has completely stopped the cadaver is placed into a clinical waste bag and stored in the freezer, ready for removal.
7. The ferret holding room can then be thoroughly cleaned, the pens are emptied, and all the equipment is washed down with sodium hypochlorite.
8. The waste is removed and autoclaved out of the unit at 121˚C. The holding room door is then sealed and fumigated using formaldehyde.

Once completed the process can start again when required.

Acknowledgements

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A study into viable wooden enrichment objects for Syrian Hamsters

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Introduction

The need to continually explore the methods in which enrichment can be provided to our animals is a high welfare priority and part of the Refinement section of the 3Rs. It is known that due to the continual growth of their teeth, hamsters require a means by which to wear down their teeth and satisfy their natural desire to chew. Due to their foraging behaviour hamsters use their cheek pouches to store and carry movable items. This can cause health problems if wooden enrichment objects produce splinters which can become lodged in the cheek pouches. However, failing to provide a method to wear down the teeth can lead to detrimental behaviour such as bar chewing, and health issues, for example, overgrown teeth and tooth loss. The aim of this study was to take a comparison of wooden products available on the market and determine a safe and effective object to be provided for hamster enrichment.

Figure 1. Standard hamster cage setup with aspen balls available. Polysulphone cage: 60.5 x 40.6 x 20.5 cm, floor area 2017 cm^2. Suitable for group-housed hamsters under the Home Office Code of Practice.[1]

Methods and materials/animals

The animals used in this study were Syrian hamsters (Figure 1), housed in groups of up to 3 per cage.

Trial objects

- large aspen chew brick (Figure 2)
- medium aspen chew brick (Figure 2)
- aspen balls (Figure 3)

The study was conducted in an AAALAC accredited research establishment, licenced under the UK Animals (Scientific Procedures) Act 1986 (ASPA).[2]

Figure 2. The 3 sizes of aspen bricks currently available, large, medium and small.

Figure 3. Aspen balls.

The animals on the enrichment trial were part of a Safety Assessment study which was licensed under ASPA however, the trial did not involve the conduct of any additional regulated procedures. Animals were housed in accordance with UK Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes.[1] When selecting trial objects for the study, only GLP certified products were considered.

Trials: Some preliminary work was conducted on the large aspen bricks but due to the high amount of surface soiling and minimal surface damage these were deemed unsuitable for future use.

Medium Bricks versus Balls: For two weeks, medium bricks were provided to 32 cages and balls were provided to 12 cages. Only 1 trial object per cage was provided due to a limited supply of aspen balls.

Data recorded:
Daily observations

- location of trial object each morning by dividing the cage into quadrants (Figure 4)
- surface damage and soiling
- weight reduction of trial object

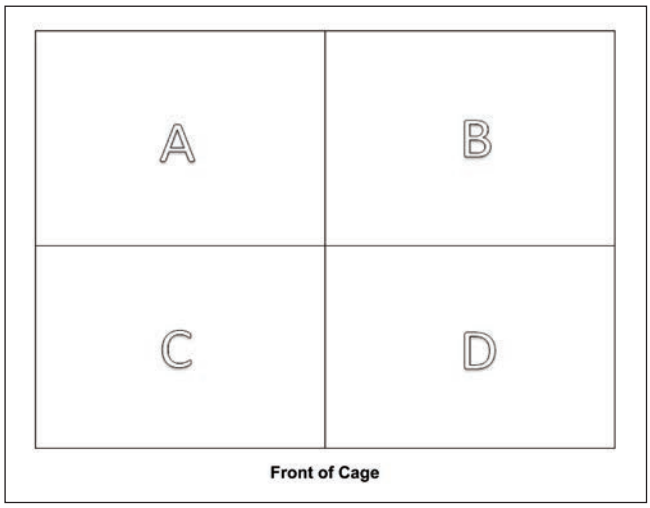

Figure 4. Example of cage quadrants.

Results

Medium Bricks

- fewer recorded movements around cage quadrants
- less surface damage and soiling
- higher remaining weight

Balls

- higher recorded movements between cage quadrants
- more surface damage and soiling
- lower remaining weight

The average percentage loss in weight of the trial objects over two weeks was significantly higher in the aspen balls than it was in the bricks (Figure 5). It was also noted that after the aspen ball had been chewed, the remains, when gently pulled away came apart in soft and short curls.

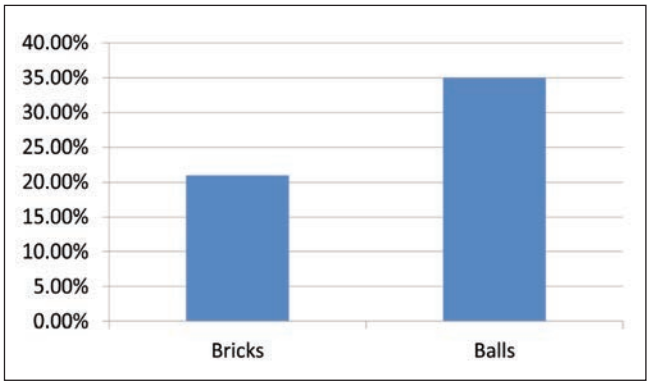

Figure 5. Average percentage loss in weight of trial objects.

The quadrant system provided some indication of how the hamsters were interacting with the trial object during the night time hours. There was an increase in the average number of cage quadrant movements when comparing the balls to the bricks (Figure 6).

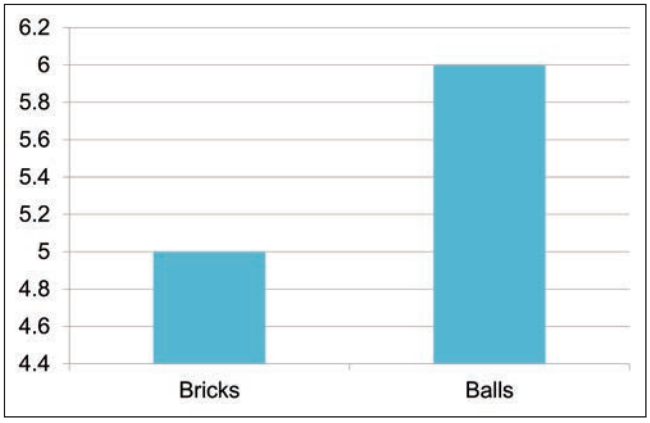

Figure 6. Average number of cage quadrant movements.

Conclusions

These results confirm that a ball shape object is a more interactive and stimulating design for hamster and the ball is thereby more frequently chewed by the hamsters (Figure 7).

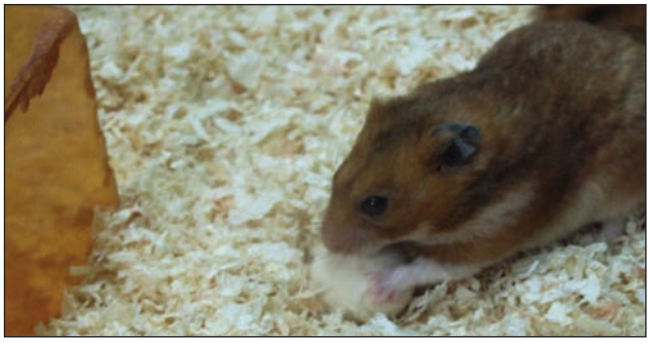

Figure 7. Hamster chewing on aspen ball.

This is a more effective method of ensuring good dental health while at the same time protecting the unique structure of the hamster's cheek pouch, due to the softer curly nature of the ball's remains. The hamsters were also observed carrying the balls around the cage when they were awake which is possible due to their smaller size and weight, this increases the objects appeal as it is able to be manipulated and moved around the cage (Figure 8).

Figure 8. Hamster carrying aspen ball around home cage.

This appeal and interest is reflected in the data by the decrease in the weight of the balls and the higher number of quadrant movements. It was decided that a minimum of 2 aspen balls per cage should be provided to group-housed hamsters to ensure equal opportunity for interaction and to reduce the chances of aggressive and dominant behaviour over a single enrichment object.

Aspen balls have proven themselves a viable and effective enrichment object for hamsters in a clinical environment.

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2 **Animals (Scientific Procedures) Act 1986** http://www.legislation.gov.uk/ukpga/1986/14/contents

Confronting crunching: a refinement for the care of mice with the desire to crunch

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Introduction

'Crunching' is the term often used to describe the abnormal behaviour of mice that habitually crunch their pelleted diet, causing substrate levels to rise as the crumbs settle on the cage floor ultimately burying the nest (Figure 1). As the science demands more and more mice to be housed in Individually Ventilated Cages (IVCs) and diets presented as compression pellets increase, the incidence of crunching is likely to rise.

Figure 1. Substrate levels rising and burying the nest.

Figure 2. Conventional use of pulp paper shelf and its application as a cruncher barrier.

When it 'comes to the crunch', the welfare and subsequent cost implications of such adverse behaviour have been difficult to tackle due to the limited options available. However, the innovative use of an inexpensive pulp paper shelf as a 'cruncher barrier' (Figure 2) not only diminishes the negative effects of crunching but also promotes nesting behaviour without completely inhibiting a mouse's desire to crunch.

Background

The nature of crunching and the influence of environmental enrichment is a disputed topic. Fiala *et al.* (1977) established that crunching may be a 'stereotypic or compulsive behaviour' because of a lack of environmental enrichment.[1] Cameron and Speakman (2010) opposed this and suggested the contrary, signifying that 'adding enrichment to cages as a strategy to reduce crunching behaviour in mice is unlikely to be successful'.[2] Furthering the dispute, crunching has been 'reported in wild rodents') and believed to be a means by which rodents discard less nutritionally valuable resources in search of a richer source.[3-5] Koteja *et al.* (2003) found that crunching could potentially be a 'heritable trait' and reported a strong correlation between siblings proposing a 'genetic influence'.[6]

Objectives

The Cruncher Barrier aims to:

Replace – destructive behaviour with a constructive one.

Reduce – diet crunching and the cost implications associated with it.

Refine – husbandry and enrichments techniques for mice with the desire to crunch.

Methods

1 – To evaluate the effectiveness of the cruncher barrier and garner the valued opinions of other Animal Technologists. 40 cruncher cages, containing 135 mice were used to assess frequency of food top-ups, base changes and nest scores. Nest scores were rated 0-3 depending on their complexity and dimension. All mice were maintained on the same diet and provided with the same environmental enrichment, subjected to daily health checks and necessary clean outs during the one week without, and one week, with a cruncher barrier.

2 – Further investigation was conducted on 12 cruncher cages of the same genetic background containing 31 mice, by measuring the daily reduction of food from the hopper for 5 nights without and 5 nights with the cruncher barrier. As a control, 12 non-cruncher cages of the same social housing structure and same genetic background were studied for 5 nights without a cruncher barrier.

Results

Nest scores

Consensus between Animal Technologists strongly indicated the cruncher barrier is beneficial in reducing crunching. As a result, animals were not disturbed as frequently due to the reduction of food top-ups and base-changes required.

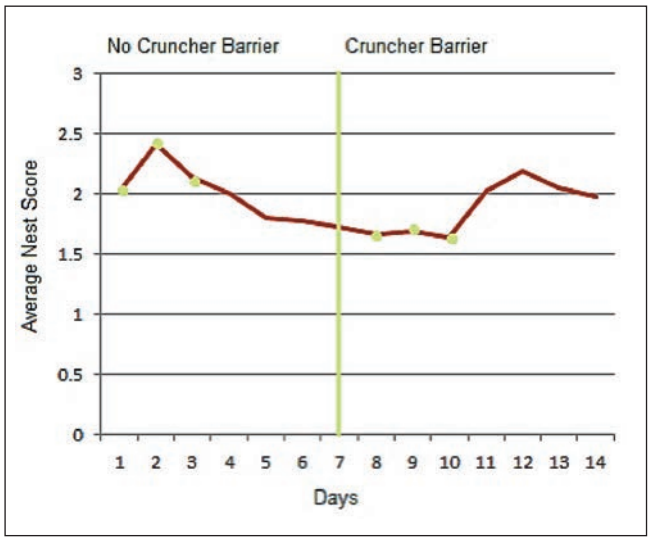

Figure 3. Fluctuation in average nest score was observed during the first three days of the week indicated by the green dots. This is believed to have occurred due to the provision of fresh bedding and nesting materials during routine base changes carried out on these days. Nest integrity and complexity gradually declines from day 3 as crunching continues. In support of our hypothesis, the application of a cruncher barrier can be seen to improve average nest scores from day 10 onwards, allowing an extended cage base change period of the cages housing cruncher mice.

Average Food Usage Via Hopper Per Mouse

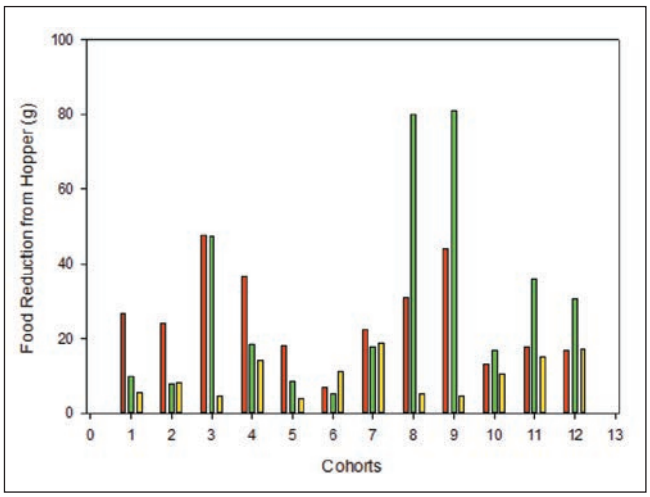

Figure 4. The preliminary results indicate that the cruncher barrier has a significant impact on crunching behaviour. For eight cages, crunching had significantly reduced per mouse, statistical analysis (Paired t-test, SigmaPlot) confirmed with a P-value of 0.031. However four cages had shown a significant increase in crunching per mouse (P=0.0372). Unpaired t-test confirmed there is a significant difference between non-crunchers and crunchers pre-cruncher barrier (P=0.0252) validating our controls. Interestingly, when comparing non-crunchers to crunchers post-cruncher barrier there was no significant difference (P=0.250) suggesting crunching had been reduced to a level considered normal. For a greater representation of the wider overall population of cruncher mice and the effect of a cruncher barrier, a new experimental design is in development.

Conclusions of implementation of the cruncher barrier

- Maintains food provisions in the hopper by reducing waste.
- Takes up minimal space in the hopper without hindering *ad libitum* feeding.
- Improves and increases environmental enrichment for cruncher mice by providing an alternative source of nesting material.
- Enhances longevity of housing conditions, providing a more sustainable lifestyle and a refinement in the care of cruncher mice.
- Economic to implement, both in materials and labour.

Acknowledgements

My thanks go to all the Animal Technologists at NHH BSU for assisting in the collection of data and their valued opinions. Many thanks also to Lawrence Moon for help with statistical analysis.

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The cotton rat – a new challenge

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Introduction

The Pharmacology department at Envigo were engaged to perform an infective study in the Cotton Rat. This presented our technologists with a whole new range of challenges in handling, husbandry and procedures. This poster explains how we learnt to adapt our current practices and procedures to suit the Cotton rat.

Background

Respiratory viral infection in humans is a great health concern, which can result in disease, death and economic losses. Cotton rats (*Sigmodon hispidus*) have been particularly useful in the study of the pathogenesis of human respiratory virus infections including the development and testing of antiviral compounds and vaccine.

History

Scientific name of the Cotton Rat is *Sigmodon hispidus*.

In the wild, cotton rats can be found over the Southern United States of America (USA), Mexico and Central America. Historically they were utilised as animal models for various human and rodent pathogens.

They were first used by the National Institute of Health (NIH) in 1937 for polio research, and since used to develop typhus vaccine and dental caries research.

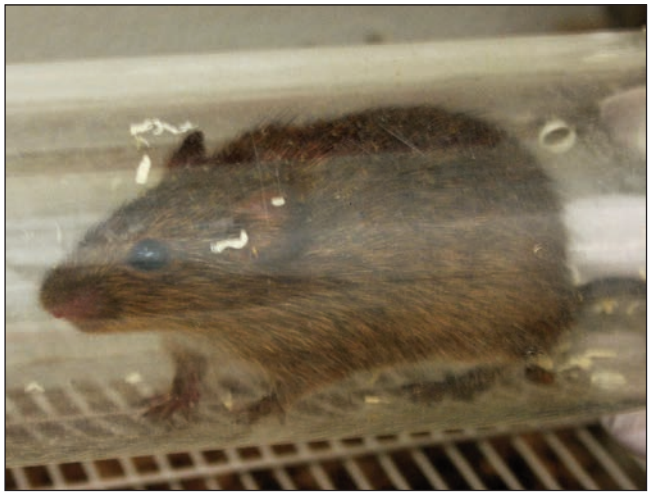

Figure 1. Cotton Rat in clear Perspex handling tube.

Research applications

Primarily used in infectious disease and immunology research although currently not very common in the UK, they have been used with the recent Zika virus outbreak. They have also been used in research on infectious disease such as Polio, Typhus, Measles and Tuberculosis (TB).

Vaccine research includes:
- Respiratory Syncytial Virus
- Genital herpes infection
- HIV
- SARS
- Influenza
- Zika virus

Cotton rat biology

- Adults can weigh 100 – 225g.
- Females sexually mature at 5 weeks.
- The gestation period is about 27 days with a litter size of around five.

Figure 2. Cotton rat in cardboard 'fun' tunnel used for nesting, etc.

– They have precocious young.
– With an average life span in the wild of 6 months but have been known to live up to 23 months in captivity.

Behaviour

– Cotton Rats have retained many of their wild characteristics.
– They tend to bite – and not let go……Ouch!
– They have a large fight or flight zone and panic when handled.
– They are predisposed to jumping, 1 metre straight up from standing start is achievable!
– They will de-glove their tails to escape.
– They are generally very timid and will 'play dead' when scared.
– Females are social animals whereas the males tend to fight.

Housing, husbandry and procedures at Envigo

We Imported 33 Female Cotton Rats to Huntingdon from our Envigo RMS site USA. Our animals were delivered at approximately 70g at 5-6 weeks of age and gained about 7g per week.

Figure 3. Cotton Rat housing at Envigo.

Husbandry and environment

Being females, we housed them together in standard P2000 Tecniplast rat caging. Microchips were injected subcutaneously under anaesthesia for identification and body temperatures. As with other laboratory rats a light cycle of 12 hours light/12 hours dark was provided with the temperature set at 22 degrees C ±2 degrees C with humidity set at 40% – 70%. Envigo 2014c pelleted rodent diet was given *ad libitum* along with *ad libitum* access to water.

Bedding and nesting material were changed twice weekly.

The animals because of their timid nature had to be weighed in pre-weighed handling tubes.

Environmental enrichments

Environmental enrichment was considered to be very important and we added a variety of items to their home cage, these included: woodflake bedding cardboard tunnels, small plastic dog chews, shredded paper (a nesting material which we found they love to snuggle together in).

We also give as food supplements including a dietary gel boost, a variety of sweet breakfast cereals, moistened rodent diet, grapes and apples – a selection of each was offered daily in a small bowl and they loved it!

Figure 4. Food supplements.

Special husbandry conditions

Due to their timid nature and difficulty in handling them we found it preferable, if not the only way, to move the animals for procedures was using a container rather than by hand. The holding containers always have lids and holes for ventilation. We very quickly realised that

Figure 5. Perspex handling tube and cardboard 'fun' tunnel.

the home cages had to be opened inside a deep container, allowing the animals to jump out and then be quietly coaxed them into cardboard or Perspex tubes. Perspex tubes allowed for visual appraisal. If we had to open the cage while it was *in situ* on the rack this needed to be done with great care as the startled cotton rat would very quickly jump through the gap resulting in an escaped animal which was then difficult to recapture.

Figure 6. Ventilated handling chamber.

Figure 7. View of home cage.

Figure 8. Handling of Cotton Rats – note depth of container required to avoid escapees.

Health

Cotton Rats are a relatively problem-free species, we saw no clinical of illness signs but they will however show signs of illness similar to those of other rodents. They can demonstrate stereotypical behaviour; this was observed in just one of our animals and remedial action was taken to alleviate this by Introducing additional 'interest' into the cage – for example sweet treats such as breakfast cereal suspended from the cage lid on a plastic cable.

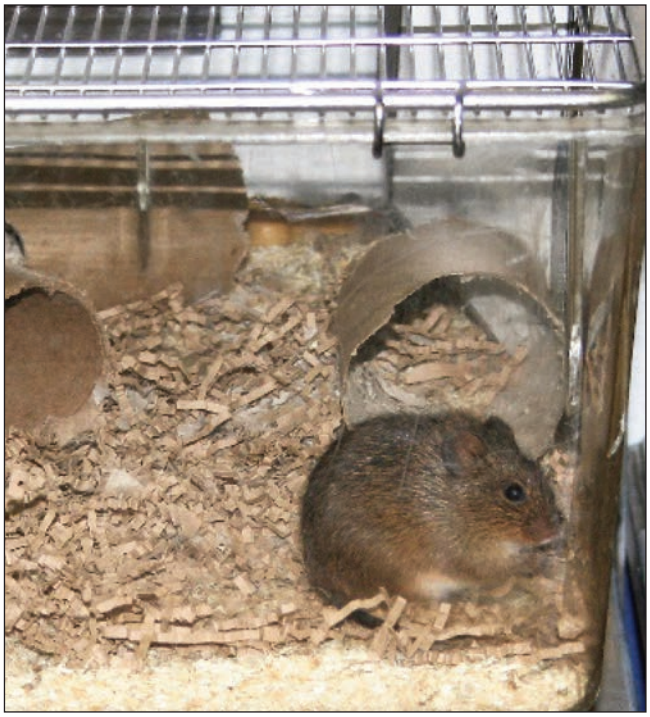

Figure 9. Cotton Rat enjoying a treat.

Dosing, blood sampling routes and anaesthesia

Our animals were dosed by intramuscular injection into the thigh muscle under isoflurane anaesthesia. The toxicokinetic blood sampling was performed using the

Figure 10. Home cage cleaned out and ready for 'house mates' to be returned.

sublingual blood vessel under Isoflurane anaesthesia however we did find that the animals salivated far more than we expected and we will be researching other anaesthetic agents for future use.

Terminal blood sampling was via cardiac puncture which was performed under anaesthesia with death being confirmed by permanent cessation of circulation. Euthanasia can also be performed using a rising concentration of carbon dioxide (CO_2) or an overdose of anaesthetic.

Conclusion

Having worked on this study we now feel that we have a much greater understanding of the Cotton Rat and how they need to be looked after and as such feel very confident that we could work with larger numbers and perform a wider range of techniques and procedures.

Does relative humidity affect reproducibility of animal research?

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Collaborators on these studies have no affiliation or financial links to SCANBUR A/S

Background

Ongoing studies show interesting preliminary data on rodent welfare and physiology when relative humidity is locally, accurately controlled at 55% (with an accuracy of ±3%) compared to when relative humidity is controlled centrally and thus fluctuates with the variable weather conditions.

Observations

At the University of Turku data collected in the calendar year 2018 on 246 breeding pairs, 628 litters and 3970 born pups of genetically altered mouse strains showed a **significant reduction in pre-weaning mortality** when relative humidity was controlled at 55% compared to building controlled.

In a facility in the United Kingdom (UK), **rat breeding pairs** housed under controlled relative humidity of 55% **produced much larger litters** compared to when they were housed under conditions where humidity levels were controlled centrally and fluctuated.

Ongoing research

In a test study in Austria, **aggression in male mice dropped** when relative humidity was controlled at 55%. Further studies are currently running.

A current study in a UK mouse facility is looking at the effects of improved control of environmental conditions on **breeding parameters in mice.** The study is looking at controlled relative humidity of 55% compared to building controlled. Publication due to be released Q22019.

A mouse facility in Canada that experiences low humidity levels during the cold winter months had challenges with **scaly skin on the mouse tails.** These health issues quickly improved, when the relative humidity was controlled at 55%.

Due to customer anecdotes suggesting improved results a study commenced in the UK to investigate the **effect** of relative humidity controlled at 55% on **Embryo Transfer in mice.** This study commenced in November 2018.

In a number of research collaborations, we are documenting the impact of accurately controlled relative humidity on reproducibility, breeding and animal welfare.

In a UK facility when tightly controlling relative humidity at different levels within the regulatory requirements the **amount of water mice drank changed significantly** in response to changes in relative humidity and was less variable compared to mice housed under room controlled relative humidity.

Mirror, mirror, on the wall

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The problem

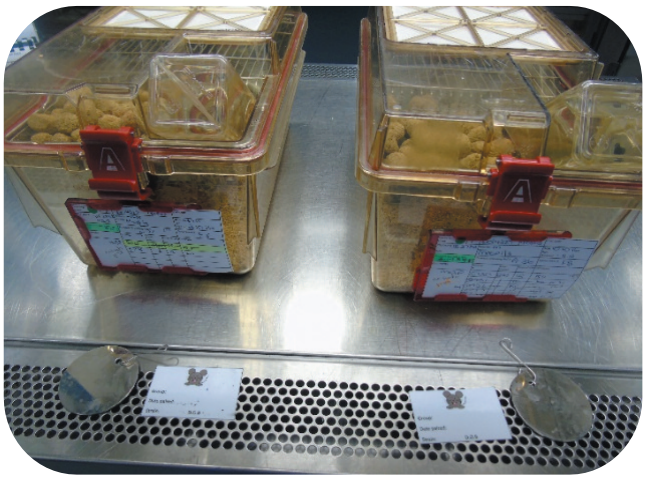
- Single housed male mice are a considerable problem in all facilities because as an industry we want to move away from keeping mice alone.
- Mice are a social species and as such we should be taking every opportunity we can to group house them but when males keep fighting each other that is easier said than done.
- The mirror method hopes to encourage single housed male mice to be grouped with others without any fighting.

The perception

- When mice are grouped together for the first time it is a novel experience filled with new sights and smells which will stress the animal too much and may cause aggression.
- It has been proven that mice can see a reflection of another mouse in the mirror so by adding a mirror prior to grouping, they become accustomed to the sight of another mouse.
- Scents cling to bedding and nesting material so swapping over both between mice being grouped also allows for them to be habituated to each other's smells.
- By the time the mice are introduced they should be used to the sights and smells of another animal reducing stress and the likelihood of fighting.

The method

Step 1



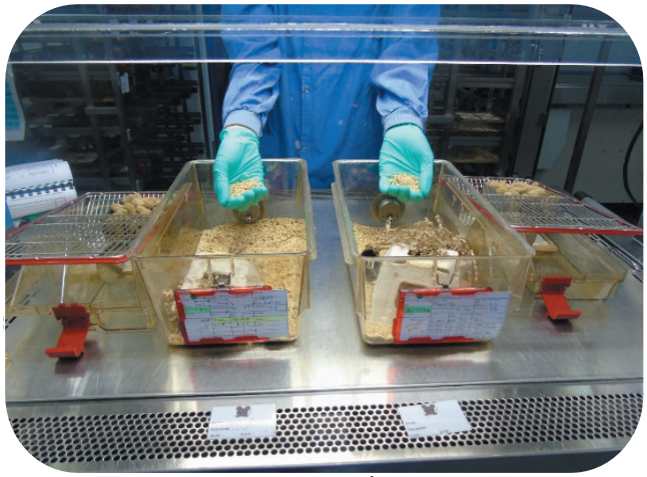
Two single housed males of a similar age are selected.

Step 2

A mirror is placed inside each cage hanging from the grid using a hook. It should be in a central position.

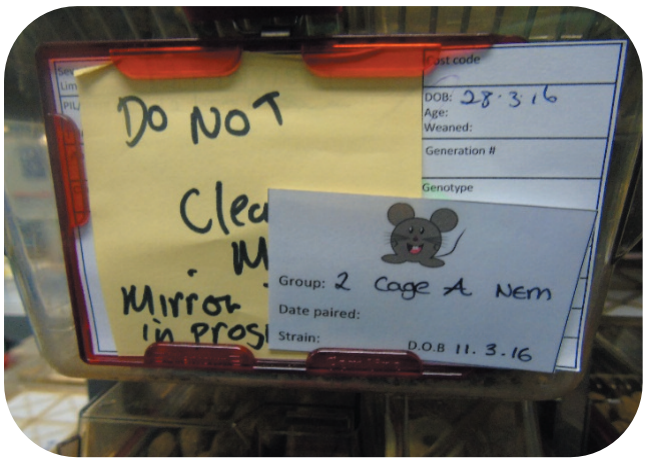
Step 3

A handful of bedding and nesting material should be swapped over daily and placed by the mirror. This should be repeated for 2-3 days.

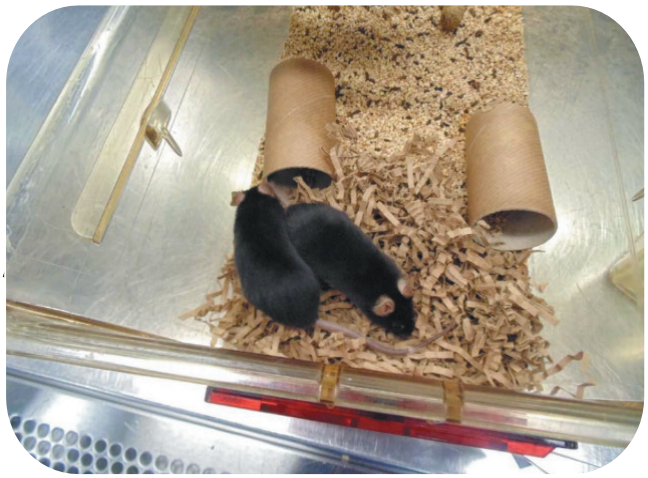


Step 4

On the final day the mice should be placed together in a clean cage with no mirror. Their cage should be left off of the rack and observed regularly on the first day.

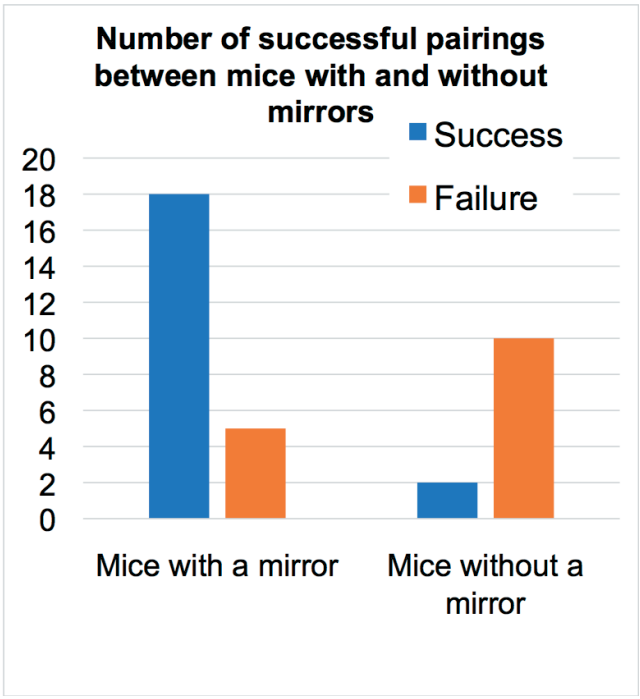


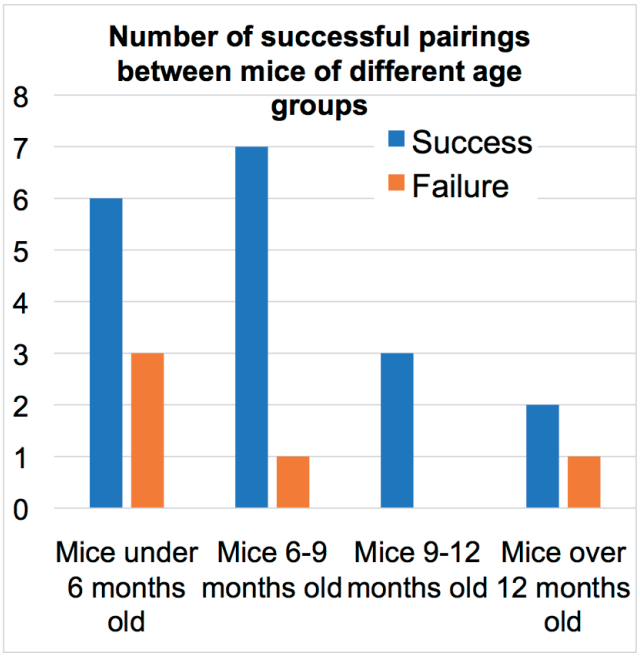
Result



The mice should now live with each other, co-existing without any signs of aggression.

The results





Hints and tips

- Though this method is successful it is not guaranteed.
- Observation skills are critical during the start of the pairing.
- Any signs of fighting should lead to mice being split.
- This method can be re-tried on them at a later date.
- The cage should not be cleaned during the first week.

- This method currently has a 78% success rate and has been used across a variety of different strains including different strain pairing.
- The mirror has been proven to be a vital part of the strategy as only 16% of non-mirror pairings were successful.
- It is also shown that the age of the mice does not make a difference so this method can be used on virtually any single mouse.
- Using this method can **refine** animal research allowing more animals to be grouped and **reduce** the number of mice that are culled for being single-housed.
- More work will be done to refine this method but if adopted, it may help make a reduction in this industry's single housed male problem.